Potency, Selectivity, and Comparative Platelet and Vascular Activity of Ralinepag Acting on Prostacyclin Receptors in Human Tissues

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Background

- Currently, prostacyclin and its analogs are widely used in the clinical management of pulmonary arterial hypertension (PAH) and are generally considered the most effective targeted PAH therapy[1]
- In PAH, the beneficial effects of prostacyclin in pulmonary arteries include vasodilation and inhibition of platelet aggregation/thrombosis, cell proliferation, and inflammation. These are thought to be mediated by binding to the prostacyclin (IP) receptor on smooth muscle cells and platelets. IP receptor binding triggers the activation of intracellular signaling resulting in increased intracellular cyclic adenosine monophosphate (cAMP)
- Ralinepag is an oral, potent and selective IP receptor agonist in development for pulmonary arterial hypertension (PAH)[1,2]

Objective

- The aim of the study was to compare the functional potency at the IP receptor (cAMP) with functional platelet and vascular effects of ralinepag with other non-prostanoid and prostacyclin mimetics (selexipag and iloprost) already licensed for PAH

Methods

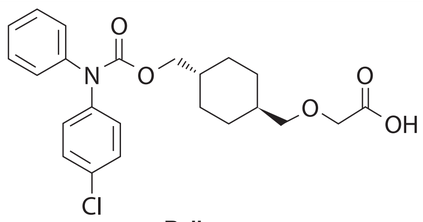
- Human IP receptor binding and selectivity was determined in recombinant IP, DP1, EP1, EP2, EP3, EP4, and TP receptor-expressing Chinese hamster ovary (CHO-K1) cells and normal human pulmonary artery smooth muscle cells (PASMCs)
- Functional potency assays, including cAMP accumulation and cell proliferation, were conducted in human PASMCs from patients with PAH
  - cAMP accumulation was also examined in recombinant CHO-K1 cells
- Distal PASMCs were isolated from the lungs of patients with Group 1 PAH (idiopathic or associated with small cardiac defects)
- Human pulmonary arteries (PAs) were derived from histologically normal tissue of patients with cancer undergoing lobectomy
  - Distal PAs were mounted in a myograph and preconstricted with U46619 (thromboxane mimetic)
- Human platelet responses were measured by light transmittance aggregometry

Results

Ralinepag Binding Affinity and Selectivity

- Ralinepag had high binding affinity (K_i=3.4 nM) and selectivity at the human IP receptor (42- to >2900-fold greater vs other prostanoid receptors) (**Figure 1**)

Figure 1. Ralinepag: Structure, Binding Affinity, and Selectivity



Ralinepag
Molecular weight: 431.9 g/mol

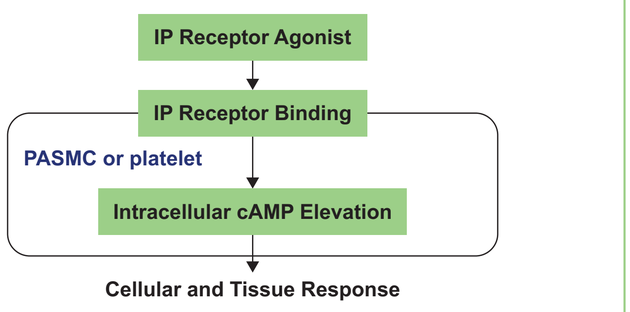
Receptor	Binding affinity K_i (nM)	Fold Selectivity
IP	3.4	1
DP_1	2610	770
EP_1	9600	2800
EP_2	610	180
EP_3	143	42
EP_4	678	200
TP	>10,000	>2900

DP_1, prostaglandin D_2 receptor 1; EP_1, prostaglandin E_2 receptor 1; EP_2, prostaglandin E_2 receptor 2; EP_3, prostaglandin E_2 receptor 3; EP_4, prostaglandin E_2 receptor 4; IP, prostacyclin receptor; TP, thromboxane receptor.

Ralinepag Has Favorable IP Receptor Activity *In Vitro*

- Iloprost, ralinepag, and MRE-269 (selexipag metabolite) increased cAMP in IP receptor–expressing CHO cells (half-maximal effective concentration [EC_{50}] 3.3, 24, and 151 nM, respectively), with similar potency in primary human PASMCs (**Figure 2**)

Figure 2. Ralinepag Is a Strong Partial Receptor Agonist of IP Receptors



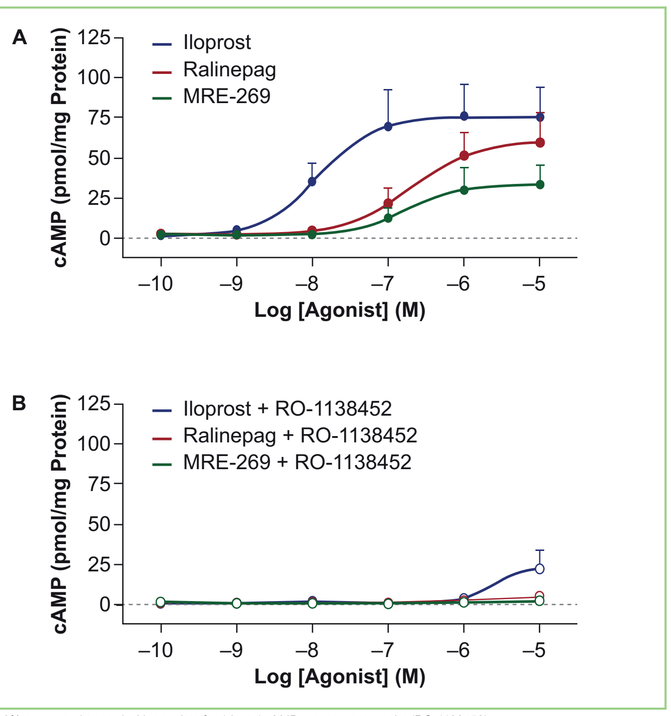
	Metric	Iloprost (i.v. use)	Ralinepag (oral use)	MRE-269* (oral use)
			Nonprostanoid	
Recombinant IP Receptor Binding (K_i)		3.4 nM	3.4 nM	3.5 nM
Functional G-protein Activation (cAMP)	CHO-K1recombinant IP receptor potency (EC_{50})	3.3 nM	24 nM	151 nM
	Primary human PASMC potency (EC_{50})	2.9 nM	24 nM	184 nM
	Efficacy	100%	67%	48%
Extent of Receptor Agonism		Full	Strong partial	Weak partial

*MRE-269 is the active metabolite of selexipag (oral).
cAMP, cyclic adenosine monophosphate; EC_{50}, half-maximal effective concentration; i.v., intravenous; PASMC, pulmonary artery smooth muscle cell.
Arena Pharmaceuticals, data on file.

Ralinepag cAMP Generation in Distal PASMCs From Patients With PAH

- Compared with the efficacy percentage of the full agonist iloprost, ralinepag is a strong partial agonist and has higher maximal cAMP stimulation versus MRE-269, a weak partial agonist (67% vs 48%, respectively; **Figure 3A**)

Figure 3. Ralinepag cAMP Generation in Distal PASMCs From Patients With PAH

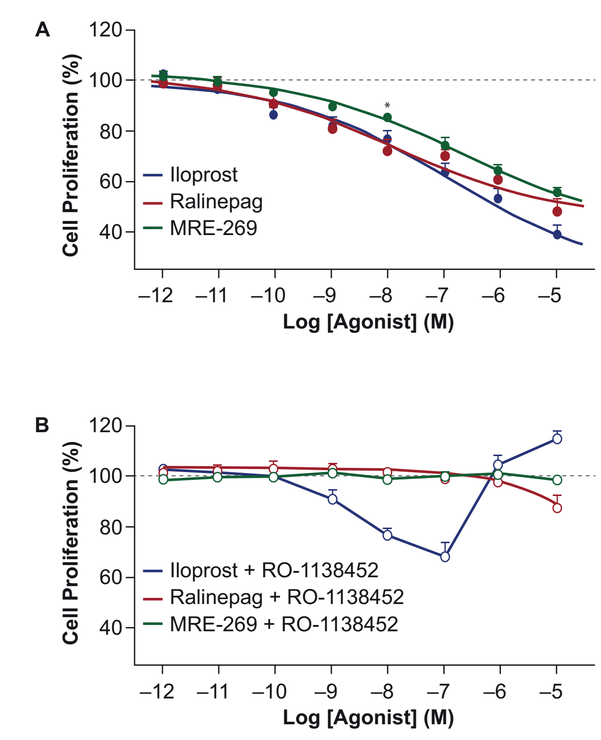


Cells grown in 9% serum and treated with agonists for 1 hr ± 1 µM IP receptor antagonist (RO-1138452).
Normalized rank order of efficacy (E_{max}): iloprost > ralinepag > MRE-269 (P<0.05).
Data are shown as mean ± standard error.
cAMP, cyclic adenosine monophosphate; PAH, pulmonary artery hypertension; PASMC, pulmonary artery smooth muscle cell.

Ralinepag Inhibition of Cell Proliferation

- In cell proliferation assays, ralinepag was tenfold more potent than MRE-269 at inhibiting PASMC proliferation (half-maximal inhibitory concentration [IC_{50}] 14 nM vs 155 nM, respectively; **Figure 4A**)
- The IP receptor antagonist RO-1138452 (1 µM) fully blocked cAMP production and cell proliferation by ralinepag and MRE-269, but only weakly inhibited or had varied effects on higher concentrations of iloprost (**Figure 3B, 4B**)

Figure 4. Cell Proliferation Assessed by the MTS Assay

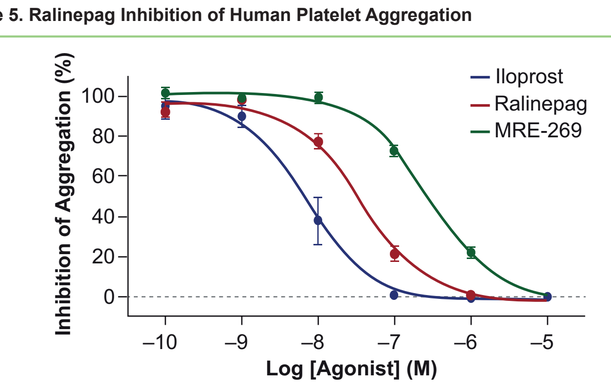


PASMCs from PAH patients grown in 9% serum and treated with agonists ± RO-1138452 for 96 hours
Data are shown as mean ± standard error. *P<0.05 (two-way analysis of variance with Bonferroni *post hoc* test) when compared with ralinepag.
MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium.

Ralinepag Has Potent Anti-platelet Activity

- Ralinepag inhibited human adenosine diphosphate (ADP)-stimulated platelet aggregation (IC_{50} 40 nM vs 288 nM) more potently than MRE-269 (**Figure 5**)

Figure 5. Ralinepag Inhibition of Human Platelet Aggregation

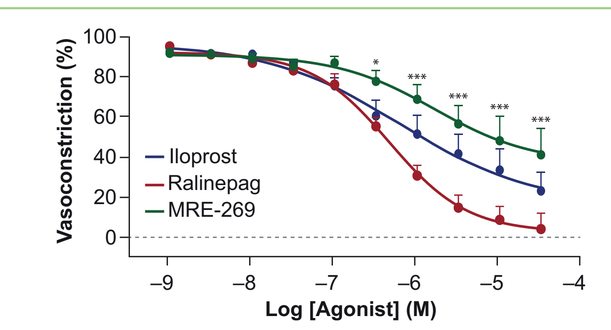


Data are shown as mean ± standard error.

Ralinepag Vascular Relaxation

- In PAs, ralinepag caused greater relaxation (E_{Max} 98%) than iloprost and MRE-269 (E_{Max} 84% and 59%); it was more potent than MRE-269 (EC_{50} 449 nM vs 1579 nM) but similar in potency to iloprost (EC_{50} 650 nM; **Figure 6**)

Figure 6. Vascular Relaxation Assessed Via Wire Myography



Human distal PAs preconstricted with a 100 nM U46619 (thromboxane mimetic).
Data are shown as mean ± standard error. *P<0.05, ***P<0.001 (two-way analysis of variance with Bonferroni post hoc test) when compared with ralinepag. PA, pulmonary artery.

Conclusions

- **Ralinepag is a potent IPRA with robust pharmacological responses in human platelets, PAs, and PASMCs**
- **The IP receptor solely accounts for cAMP production and the antiproliferative effects of ralinepag and selexipag metabolite, but not iloprost**
- **Ralinepag demonstrated greater functional potency and efficacy than the active selexipag metabolite, MRE-269, and a favorable vasorelaxant profile compared with iloprost and MRE-269, providing rationale for further investigation of ralinepag in PAH**

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Disclosures

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References

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